

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OF PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A method for the *in vitro* micropropagation of a phytopharmaceutical plant comprising:
 - a) culturing a sterile explant of said phytopharmaceutical plant on an induction medium comprising at least one plant growth regulator having cytokinin activity, to form regenerated tissue; and
 - b) transferring said regenerated tissue to a basal medium and culturing to form plantlets.
2. The method of claim 1 wherein said phytopharmaceutical plant is selected from the group consisting of:
 - Achillea millefolium
 - Achyranthes bidentata
 - Aconitum napellus
 - Adonis aestivalis
 - Agastache mexicana
 - Agrimonia eupatoria
 - Agathosma betulina
 - Allium sp
 - Anchusa officinalis
 - Anemopsis californica
 - Angelica dahurica
 - Angelica polymorpha sinensis (A. sinensis)
 - Arnica Montana
 - Ammi visnaga
 - Arctostaphylos uva-ursi

Asclepias tuberosa
Astragalus membranaceus
Astragalus chinensis
Baphicacanthus cusia
Bixa orellana
Bupleurum falcatum
Brugmansia (Datura) spp.
Campanula rapunculus
Carum roxburgianum
Carum copticum
Cassia tora
Chamaelirium luteum
Chimaphila umbellata
Commiphora africana
Conium maculatum
Crithium maritimum
Datura metel (Datura alba)
Datura inoxia
Dracocephalum moldavica
Echinacea sp.
Eclipta alba (E. prostrata)
Ephedra nevadensis
Eriodictyon californicum
Eucommia ulmoides
Eupatorium perfoliatum
Filipendula vulgaris (F. hexapetala)
Gaultheria procumbens
Geum urbanum

Houttuynia cordata
Hydrocotyle asiatica (Centella asiatica)
Hypericum perforatum cv. Anthos
Inula helenium
Jatropha curcas
Leptospermum scoparium
Lespedeza capitata
Ligusticum porteri
Ligustrum lucidum
Lithospermum officinale
Lycium barbarum
Mucuna pruriens
Mandragora officinarum
Origanum dictamnus
Parietaria judaica (P. officinalis)
Phyllanthus emblica
Picrasma excelsa
Piniella ternate
Pogostemon patchouli
Polygonum multiflorum
Porophyllum ruderale ssp. macrocephalum
Prunella vulgaris
Pueraria lobata (P. thunbergiana)
Rauvolfia serpentina
Rivea corymbosa
Sanguinaria Canadensis
Satureja douglasii
Schizonepeta tenuifolia

Scutellaria baicalensis
Solanum xanthocarpum (S. surattense)
Sutherlandia frutescens
Tabebuia impetiginosa
Tanacetum parthenium
Tribulus terrestris
Trichosanthes kirilowii
Turnera diffusa
Voacanga africana, and
Withania somnifera

3. The method according to claim 2, wherein said phytopharmaceutical plant is selected from St. John's wort (*Hypericum perforatum* cv. Anthos), Huang-qin (*Scutellaria baicalensis*), *Echinacea* sp. and feverfew (*Tanacetum parthenium*).
4. The method according to claim 1, wherein said at one plant growth regulator having cytokinin activity is selected from the group consisting of thidiazuron (TDZ, *N*-phenyl-*N'*-(1,2,3-thiadiazol-yl)urea), benzylaminopurine (BAP), zeatin, CPPU (N-(2-chloro-4pyridyl)-N(-phenyl urea) and 2-*i*-P (N6-(2-isopentenyl) adenine or 6-gamma,gamma-dimethylallylamino purine).
5. The method according to claim 4, wherein said at least one plant growth regulator having cytokinin activity is selected from thidiazuron (TDZ) and benzylaminopurine (BAP).
6. The method according to claim 5, wherein said induction medium comprises from about 0.001 to about 25 $\mu\text{mol} \cdot \text{L}^{-1}$ of said at least plant growth regulator having cytokinin activity.

7. The method according to claim 5, wherein said sterile explant is maintained on said induction medium from about 1 to about 50 days.
8. The method according to claim 1, wherein said explant is selected from the seed, petiole, hypocotyl, stem, cotyledon and leaf.
9. The method according to claim 1, wherein said phytopharmaceutical plant is St. John's wort.
10. The method according to claim 9, wherein said plant growth regulator having cytokinin activity is thidiazuron.
11. The method according to claim 10, wherein the induction medium comprises thiadiazuron from about 0.001 to about $25 \mu\text{mol} \cdot \text{L}^{-1}$.
12. The method according to claim 11, wherein the induction medium comprises thiadiazuron from about 4 to about $10 \mu\text{mol} \cdot \text{L}^{-1}$.
13. The method according to claim 9, wherein said sterile explant is maintained on said induction medium from about 1 to about 15 days.
14. The method according to claim 13, wherein said sterile explant is maintained on said induction medium from about 8 to about 10 days.
15. The method according to claim 9, wherein said explant is etiolated hypocotyl.
16. The method according to claim 1, wherein the phytopharmaceutical plant is *Echinacea sp.*.

17. The method according to claim 16, wherein said plant growth regulator having cytokinin activity is selected from the group consisting of thidiazuron and benzylaminopurine.
18. The method according to claim 17, wherein said induction medium comprises from about 0.001 to about 25 $\mu\text{mol} \cdot \text{L}^{-1}$ of said plant growth regulator having cytokinin activity.
19. The method according to claim 18, wherein said plant growth regulator having cytokinin activity is from about 1.0 to about 15 $\mu\text{mol} \cdot \text{L}^{-1}$.
20. The method according to claim 16, wherein said sterile explant is maintained on said induction medium from about 1 to about 50 days.
21. The method according to claim 20, wherein said sterile explant is maintained on said induction medium from about 10 to about 35 days.
22. The method according to claim 16, wherein said explant is petiole.
23. The method according to claim 1, wherein said phytopharmaceutical plant is Huang qin.
24. The method according to claim 23, wherein said plant growth regulator having cytokinin activity is thidiazuron.
25. The method according to claim 24, wherein said induction medium comprises from about 0.001 to about 25 $\mu\text{mol} \cdot \text{L}^{-1}$ of said plant growth regulator having cytokinin activity.

26. The method according to claim 25, wherein said plant growth regulator having cytokinin activity is from about 1.5 to about $20 \mu\text{mol} \cdot \text{L}^{-1}$
27. The method according to claim 23, wherein said sterile explant is maintained on said induction medium from about 1 to about 30 days.
28. The method according to claim 27, wherein said sterile explant is maintained on said induction medium from about 14 to about 20 days.
29. The method according to claim 23, wherein said explant is selected from seeds, hypocotyl and stems.
30. The method according to claim 1, wherein the phytopharmaceutical plant is feverfew.
31. The method according to claim 30, wherein said plant growth regulator having cytokinin activity is thidiazuron.
32. The method according to claim 31, wherein said induction medium comprises from about 0.001 to about $25 \mu\text{mol} \cdot \text{L}^{-1}$ of said plant growth regulator having cytokinin activity.
33. The method according to claim 32, wherein said plant growth regulator having cytokinin activity is from about 2.0 to about $8.0 \mu\text{mol} \cdot \text{L}^{-1}$
34. The method according to claim 30, wherein said sterile explant is maintained on said induction medium from about 1 to about 50 days.

35. The method according to claim 34, wherein said sterile explant is maintained on said induction medium from about 20 to about 35 days.

36. The method according to claim 30, wherein the explant is selected from leaf, stem, petiole and hypocotyl.

37. A method for the *in vitro* micropropagation of a phytopharmaceutical plant comprising:

a) culturing a sterile explant of said phytopharmaceutical plant on an induction medium comprising a suitable concentration of at least one plant growth regulator having cytokinin activity, to form regenerated tissue;

b) transferring said regenerated tissue to a basal medium and subculturing to allow optimized formation of regenerated tissue; and

c) placing said regenerated tissue on a basal medium and culturing to form plantlets.

38. The method according to claim 37, wherein said phytopharmaceutical plant is selected from the group consisting of:

Achillea millefolium

Achyranthes bidentata

Aconitum napellus

Adonis aestivalis

Agastache mexicana

Agrimonia eupatoria

Agathosma betulina

Allium sp

Anchusa officinalis

Anemopsis californica

Angelica dahurica
Angelica polymorpha sinensis (A. sinensis)
Arnica Montana
Ammi visnaga
Arctostaphylos uva-ursi
Asclepias tuberosa
Astragalus membranaceus
Astragalus chinensis
Baphicacanthus cusia
Bixa orellana
Bupleurum falcatum
Brugmansia (Datura) spp.
Campanula rapunculus
Carum roxburgianum
Carum copticum
Cassia tora
Chamaelirium luteum
Chimaphila umbellata
Commiphora africana
Conium maculatum
Crithium maritimum
Datura metel (Datura alba)
Datura inoxia
Dracocephalum moldavica
Echinacea sp.
Eclipta alba (E. prostrata)
Ephedra nevadensis
Eriodictyon californicum

Eucommia ulmoides
Eupatorium perfoliatum
Filipendula vulgaris (F. hexapetala)
Gaultheria procumbens
Geum urbanum
Houttuynia cordata
Hydrocotyle asiatica (Centella asiatica)
Hypericum perforatum cv. Anthos
Inula helenium
Jatropha curcas
Leptospermum scoparium
Lespedeza capitata
Ligusticum porteri
Ligustrum lucidum
Lithospermum officinale
Lycium barbarum
Mucuna pruriens
Mandragora officinarum
Origanum dictamnus
Parietaria judaica (P. officinalis)
Phyllanthus emblica
Picrasma excelsa
Piniella ternate
Pogostemon patchouli
Polygonum multiflorum
Porophyllum ruderale ssp. macrocephalum
Prunella vulgaris
Pueraria lobata (P. thunbergiana)

Rauvolfia serpentina
Rivea corymbosa
Sanguinaria Canadensis
Satureja douglasii
Schizonepeta tenuifolia
Scutellaria baicalensis
Solanum xanthocarpum (S. surattense)
Sutherlandia frutescens
Tabebuia impetiginosa
Tanacetum parthenium
Tribulus terrestris
Trichosanthes kirilowii
Turnera diffusa
Voacanga africana, and
Withania somnifera

39. The method according to claim 38, wherein the phytopharmaceutical plant is selected from the group consisting of St. John's wort (*Hypericum perforatum* cv. Anthos), Huang-qin (*Scutellaria baicalensis*), *Echinacea* sp. and feverfew (*Tanacetum parthenium*).

40. The method according to claim 37, wherein said sterile explant of a phytopharmaceutical plant is grown on a medium comprising one plant growth regulator having cytokinin activity which is selected from thidiazuron (TDZ, *N*-phenyl-*N'*-(1,2,3-thidiazol-yl)urea), benzylaminopurine (BAP), zeatin, CPPU (*N*-(2-chloro-4pyridyl)-*N'*-(phenyl urea) and 2-*i*-P (*N*6-(2-isopentenyl) adenine or 6-gamma,gamma-dimethylallylamino purine).

41. The method according to claim 40, wherein said plant growth regulator having cytokinin activity is selected from the group consisting of thidiazuron (TDZ) and benzylaminopurine (BAP).

42. The method according to claim 41, wherein, in said culturing step, said induction medium comprises from about 0.001 to about 25 $\mu\text{mol} \cdot \text{L}^{-1}$ of said plant growth regulator having cytokinin activity.

43. The method according to claim 41, wherein, in said culturing step, said sterile explant is maintained on said induction medium from about 1 to about 50 days.

44. The method according to claim 37, wherein, in said culturing step, said explant is selected from the seed, petiole, hypocotyl, stem, cotyledon and leaf.

45. The method according to claim 37, wherein, in said transferring step, said regenerated tissue is subcultured for about 1 to about 15 days.

46. A method for phytofortification of an *in vitro*-grown phytopharmaceutical plant comprising:

a) culturing a sterile seedling, explant or regenerated tissues to form a plantlet;

and

b) subculturing said plantlet onto a basal medium containing at least one additive of interest, to allow uptake and accumulation of said at least one additive of interest in a bio-available form in said plantlet.

47. The method according to claim 46, wherein the phytopharmaceutical plant is selected from the group consisting of:

Achillea millefolium

Achyranthes bidentata
Aconitum napellus
Adonis aestivalis
Agastache mexicana
Agrimonia eupatoria
Agathosma betulina
Allium sp
Anchusa officinalis
Anemopsis californica
Angelica dahurica
Angelica polymorpha sinensis (A. sinensis)
Arnica Montana
Ammi visnaga
Arctostaphylos uva-ursi
Asclepias tuberosa
Astragalus membranaceus
Astragalus chinensis
Baphicacanthus cusia
Bixa orellana
Bupleurum falcatum
Brugmansia (Datura) spp.
Campanula rapunculus
Carum roxburgianum
Carum copticum
Cassia tora
Chamaelirium luteum
Chimaphila umbellata
Commiphora africana

Conium maculatum
Crithium maritimum
Datura metel (Datura alba)
Datura inoxia
Dracocephalum moldavica
Echinacea sp.
Eclipta alba (E. prostrata)
Ephedra nevadensis
Eriodictyon californicum
Eucommia ulmoides
Eupatorium perfoliatum
Filipendula vulgaris (F. hexapetala)
Gaultheria procumbens
Geum urbanum
Houttuynia cordata
Hydrocotyle asiatica (Centella asiatica)
Hypericum perforatum cv. Anthos
Inula helenium
Jatropha curcas
Leptospermum scoparium
Lespedeza capitata
Ligusticum porteri
Ligustrum lucidum
Lithospermum officinale
Lycium barbarum
Mucuna pruriens
Mandragora officinarum
Origanum dictamnus

Parietaria judaica (P. officinalis)
Phyllanthus emblica
Picrasma excelsa
Piniella ternate
Pogostemon patchouli
Polygonum multiflorum
Porophyllum ruderale ssp. macrocephalum
Prunella vulgaris
Pueraria lobata (P. thunbergiana)
Rauvolfia serpentina
Rivea corymbosa
Sanguinaria Canadensis
Satureja douglasii
Schizonepeta tenuifolia
Scutellaria baicalensis
Solanum xanthocarpum (S. surattense)
Sutherlandia frutescens
Tabebuia impetiginosa
Tanacetum parthenium
Tribulus terrestris
Trichosanthes kirilowii
Turnera diffusa
Voacanga africana, and
Withania somnifera

48. The method according to claim 46, wherein said at least one additive is selected from the group consisting of boron, calcium, chloride, chromium, cobalt, copper, iron, lithium, iodine, magnesium, manganese, molybdenum, nickel,

phosphorous, potassium, selenium, silicon, sodium, sulphur, tin, vanadium and zinc.

49. The method according to claim 48, wherein said at least one additive of interest is zinc.

50. A method according to claim 48, wherein said at least one additive of interest is lithium.

51. The method according to claim 46, wherein said at least one additive of interest within said basal medium, is from about 0.001 to about 500 mg•L⁻¹.

52. A method according to claim 51 wherein in said step of culturing, said plantlet is maintained on said basal media from about 1 to about 50 days.

53. The method according to claim 46, wherein, in said step of culturing, said explant is selected from the group consisting of a seed, petiole, hypocotyl, stem, cotyledon, root and leaf.

54. The method according to claim 46, wherein said phytopharmaceutical plant is St. John's wort.

55. The method according to claim 46, wherein said phytopharmaceutical plant is *Echinacea sp.*

56. The method according to claim 46, wherein, in said step of culturing, said plantlets are produced either:

a) on a sterile explant of said phytopharmaceutical plant grown on an induction medium comprising at least one plant growth regulator having cytokinin

activity, or

- b) grown from a sterile seed, or
- c) seedling in culture.

57. The method according to claim 56, wherein said at least one plant growth regulator having cytokinin activity is selected from the group consisting of thidiazuron (TDZ, *N*-phenyl-*N'*-(1,2,3-thidiazol-yl)urea), benzylaminopurine (BAP), zeatin, CPPU (N-(2-chloro-4pyridyl)-N-phenyl urea) and 2-*i*-P (N6-(2-isopentenyl) adenine or 6-gamma,gamma-dimethylallylamino purine).

58. A method for the *in vivo* phytofortification of a phytopharmaceutical plant comprising:

- a) culturing a plantlet or seedling of said phytopharmaceutical plant as defined in claim 1, for clonal micropropagation and growth of said plantlet; and
- b) adaptating the plantlet or seedling to a hydroponic environment with a recycling solution containing at least one additive of interest to allow uptake and accumulation of said at least one additive of interest in a bioavailable form within said plantlet or seedling.

59. The method according to claim 58, wherein said phytopharmaceutical plant is selected from the group consisting of:

Achillea millefolium
Achyranthes bidentata
Aconitum napellus
Adonis aestivalis
Agastache mexicana
Agrimonia eupatoria
Agathosma betulina

Allium sp
Anchusa officinalis
Anemopsis californica
Angelica dahurica
Angelica polymorpha sinensis (A. sinensis)
Arnica Montana
Ammi visnaga
Arctostaphylos uva-ursi
Asclepias tuberosa
Astragalus membranaceus
Astragalus chinensis
Baphicacanthus cusia
Bixa orellana
Bupleurum falcatum
Brugmansia (Datura) spp.
Campanula rapunculus
Carum roxburgianum
Carum copticum
Cassia tora
Chamaelirium luteum
Chimaphila umbellata
Commiphora africana
Conium maculatum
Crithium maritimum
Datura metel (Datura alba)
Datura inoxia
Dracocephalum moldavica
Echinacea sp.

Eclipta alba (E. prostrata)
Ephedra nevadensis
Eriodictyon californicum
Eucommia ulmoides
Eupatorium perfoliatum
Filipendula vulgaris (F. hexapetala)
Gaultheria procumbens
Geum urbanum
Houttuynia cordata
Hydrocotyle asiatica (Centella asiatica)
Hypericum perforatum cv. Anthos
Inula helenium
Jatropha curcas
Leptospermum scoparium
Lespedeza capitata
Ligusticum porteri
Ligustrum lucidum
Lithospermum officinale
Lycium barbarum
Mucuna pruriens
Mandragora officinarum
Origanum dictamnus
Parietaria judaica (P. officinalis)
Phyllanthus emblica
Picrasma excelsa
Piniella ternate
Pogostemon patchouli
Polygonum multiflorum

Porophyllum ruderae ssp. macrocephalum
Prunella vulgaris
Pueraria lobata (P. thunbergiana)
Rauvolfia serpentina
Rivea corymbosa
Sanguinaria Canadensis
Satureja douglasii
Schizonepeta tenuifolia
Scutellaria baicalensis
Solanum xanthocarpum (S. surattense)
Sutherlandia frutescens
Tabebuia impetiginosa
Tanacetum parthenium
Tribulus terrestris
Trichosanthes kirilowii
Turnera diffusa
Voacanga africana, and
Withania somnifera

60. The method according to claim 58, wherein in said culturing step, said at least one additive of interest is selected from boron, calcium, chloride, chromium, cobalt, copper, iron, lithium, iodine, magnesium, manganese, molybdenum, nickel, phosphorous, potassium, selenium, silicon, sodium, sulphur, tin, vanadium and zinc.

61. A phytopharmaceutical plant prepared by the method of claim 46.